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(54) Title: LINEAR ARRAYS OF IMMOBILIZED COMPOUNDS AND METHODS OF USING SAME

(57) Abstract

The present invention provides spatially–addressable linear arrays of immobilized compounds, such as peptides and polynucleotides, that can be used in a wide variety of assay formats to identify target analyte compounds which interact with the immobilized compounds.

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LINEAR ARRAYS OF IMMOBILIZED COMPOUNDS AND METHODS OF USING SAME

1. CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of application Serial No. 09/083,861, filed May 21, 1998, which is a continuation-in-part of application Serial No. 08/959,362 filed October 28, 1997, which is a continuation-in-part of application Serial No. 08/947,779, filed October 9, 1997, which is a continuation-in-part of application Serial No. 08/912,885, filed August 15, 1997, which is a continuation-in-part of application Serial No. 08/892,503, filed July 14, 1997, which is a continuation-in-part of application Serial No. 08/812,951, filed March 4, 1997, which is a continuation-in-part of application Serial No. 08/784,747, filed January 16, 1997. The disclosures of all of these applications are incorporated herein by reference in their entireties. Priority under 35 USC § 120 is claimed to application Serial No. 09/083,861.

2. FIELD OF THE INVENTION

The present invention relates to apparatuses and methods useful in assays and technologies involving spatially-addressable arrays of immobilized compounds.

3. BACKGROUND OF THE INVENTION

Recently, a variety of techniques that take advantage of synthetic arrays or libraries of immobilized compounds have been developed. These methods typically involve contacting an array of immobilized compounds with a target molecule of interest to identify those compounds in the array that interact with the target (*e.g.*, bind the target). The types of synthetic arrays or libraries used in such assays generally fall into two categories: bead-based libraries and spatially-addressable arrays.

In bead-based libraries, thousands or even millions of different compounds, for example peptides or small organic molecules, are synthesized on individual beads, typically with one compound per bead, using combinatorial methods. Due to the combinatorial nature of the synthesis, the entire library is synthesized in parallel. The beads are then screened in bulk with a labeled target receptor, such as an antibody,

enzyme or other receptor, under conditions conducive to binding between the labeled receptor and immobilized compounds which are specific for the receptor. Beads which bound the receptor are then identified and the structures of the compounds immobilized thereon determined.

In first generation bead-based libraries, the structure of the compounds were determined by dissociating the receptor-compound complex, cleaving the immobilized compound from the bead and subjecting the compound to standard analytical techniques such as HPLC, mass spectroscopy, etc. However, as it was not always feasible to dissociate the complex, and oftentimes the beads did not contain adequate quantities of compound for structural analysis, second generation bead-based libraries utilizing code or tag molecules were developed. In these encoded bead libraries, the code or tag molecule is co-synthesized on the bead with the compound of interest and the structures of the compounds attached to the beads are determined by analyzing the respective code or tag molecules. Thus, following a screening assay, beads which bind the target receptor can be removed from the pool of beads and the structures of the compounds attached thereto determined by simply analyzing the code or tag molecules. A variety of code or tag molecules have been developed for use in such libraries, including oligonucleotides (Needels *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:10700-10704; Lerner *et al.*, 1992, Proc. Natl. Acad. Sci USA 89:5181-5183; WO 93/20242), peptides (Kerr *et al.*, 1993, J. Am. Chem. Soc. 115: 2529-2531; Nikolaiev *et al.*, 1993, Pept. Res. 6: 161-170) and chromatographically resolvable halocarbon derivatives (Ohlmeyer *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90: 10922-10926; Borchardt *et al.*, 1994, J. Am. Chem. Soc. 116: 373-374).

While non-encoded and encoded bead-based libraries provide a powerful improvement over traditional techniques for identifying new compounds which bind a target receptor of interest (for a review of the various types of compounds that have been identified using such libraries *see* Gallop *et al.*, 1994, J. Med. Chem. 37: 1233-1251; Gordon *et al.*, 1994, J. Med. Chem. 37:1385-1401), these methods still suffer significant drawbacks. For example, since the entire library is synthesized in parallel in a combinatorial fashion, the integrity of the library cannot be verified prior to use. Checking the fidelity of the synthesis would require analyzing the compound attached to

each bead- an enormous task for libraries comprised of even a few hundred members. The inability to verify the integrity of the library can lead to ambiguous results. It must be presumed that each compound defined by the combinatorial synthesis is represented in the library, which, due to synthesis failures, may not be the case.

In addition, not all compounds in the library are synthesized in equal quantities. Depending upon the particular reaction kinetics, certain compounds may be overrepresented. Again, this can lead to ambiguous results, as a particular compound may be produced in insufficient quantities to be detected in an assay. Moreover, since the beads are typically screened with the receptor in a batch-wise fashion, large volumes, and hence large quantities, of labeled target receptor are required for the screening assay. Quite often, the quantities of target receptor needed to screen the library are simply unavailable.

Encoded bead libraries suffer additional drawbacks. Because the code or tag molecules and compounds of interest are co-synthesized on the beads, their various protetecting schemes and synthetic chemistries must be compatible. Depending on the compounds desired for the library, in many instances suitable chemistries may not be available or may need to be developed.

In spatially-addressable libraries or arrays, the compounds of interest are attached to the surface of a flat, planar substrate, such as a glass slide, to form a two-dimensional grid or matrix of compounds The arrays are spatially-addressable in the sense that the structures of the compounds composing the array are known, and are therefore defined by their spatial addresses (*xy* coordinates). In use, the arrays are screened with a labeled target receptor and scanned for signal. Only those addresses (spots) of the array containing compounds which bound the labeled receptor produce a signal. The identities or structures of the compounds attached to these positive spots are then determined from their *xy* coordinates. Since it is advantageous to screen numerous compounds simultaneously, the arrays are typically miniaturized and contain high densities of compounds.

One array-based application that requires very high density miniaturized two-dimensional arrays is sequencing by hybridization (SBH). In one common format of SBH (format II), a spatially-addressable array of the complete set of oligonucleotide probes of

length *k* is contacted with a labeled target nucleic acid under conditions which discriminate between the formation of perfectly complementary probe-target hybrids and mis-matched probe-target hybrids. Following washing, the array is scanned for signals, the sequences of hybridized probes determined from their spatial addresses, and the sequence of the target nucleic acid determined by overlapping the common sequences of the hybridized probes.

Two other SBH formats also exist. In format I SBH, the target nucleic acid is immobilized on a solid support, *e.g.*, a nylon or nitrocellulose filter, and interrogated with solution-phase labeled probes. Typically, the target is interrogated with a single labeled probe at a time, or alternatively with a plurality of labeled probes, each of which bears a different distinguishable label (this latter mode is termed "multiplexing"). To reduce the number of manipulations required, the target nucleic acid can be spotted onto a filter in a grid or array. Each spot or address in the array is then interrogated with a single labeled probe or plurality of multiplexed labeled probes.

In yet another format of SBH (format III), two sets of probes are used: an immobilized set (in the form of an immobilized two-dimensional array) and a solution-phase set. The solution-phase probes are each labeled with a different, distinguishable label. The immobilized probes are unlabeled. In format III SBH, the array of immobilized probes is contacted with an unlabeled target nucleic acid and the labeled set of solution-phase probes under conditions which discriminate between fully complementary and mismatched probe-target complexes. The entire array is then exposed to conditions which covalently join properly aligned probes (typically by adding a DNA ligase to the assay solution). After washing away unhybridized target nucleic acid and unligated labeled probes, the array is scanned for signal. Only those positions of the array where ligation occurred produce a signal. The sequences of the ligated probes are then determined from their spatial addresses combined with the known sequence of the labeled probe and the sequence of the target nucleic acid determined by overlapping the common sequences of the ligated probes.

For a review of the three types of SBH and their respective advantages, see U.S. 5,202,231; U.S. 5,525,464; WO 98/31836; WO 96/17957 and the references cited therein.

To meet the needs of applications requiring high-density miniaturized arrays of immobilized compounds, such as SBH and its related applications, two general methods have been developed for synthesizing the immobilized arrays: *in situ* methods in which each compound in the array is synthesized directly on the surface of the substrate and deposition methods in which pre-synthesized compounds capable of being covalently attached to the surface of the substrate are deposited, typically by way of robot dispensing devices, at the appropriate spatial addresses.

In situ methods typically require specialized reagents and complex masking strategies. For example, Fodor *et al.*, 1991, Science 251:767-773 describe an *in situ* method which utilizes photo-protected amino acids and photo lithographic masking strategies to synthesize miniaturized, spatially-addressable arrays of peptides. This *in situ* method has recently been expanded to the synthesis of miniaturized arrays of oligonucleotides (U.S. Patent No. 5,744,305). Another *in situ* synthesis method for making spatially-addressable arrays of immobilized oligonucleotides is described by Southern, 1992, Genomics 13:1008-1017; *see also* Southern & Maskos, 1993, Nucl. Acids Res. 21:4663-4669; Southern & Maskos, 1992, Nucl. Acids Res. 20:1679-1684; Southern & Maskos, 1992, Nucl. Acids Res. 20:1675-1678. In this method, conventional oligonucleotide synthesis reagents are dispensed onto physically masked glass slides to create the array of immobilized oligonucleotides.

Deposition methods typically require precisely controlled robotic delivery of defined volumes of reagents. U.S. Patent No. 5,807,522 describes a deposition method for making micro arrays of biological samples that involves dispensing a known volume of reagent at each address of the array by tapping a capillary dispenser on the substrate under conditions effective to draw a defined volume of liquid onto the substrate.

One of the biggest drawbacks of both the *in situ* and deposition microfabrication techniques is the inability to verify the integrity of the array once it has been synthesized. Absent analyzing the compound immobilized at each address, the integrity of the deposition or *in situ* synthesis chemistry simply cannot be verified. Such an analysis would be extremely labor intensive, and may even be impossible for extremely high-density arrays, as the quantity of compound immobilized may not be sufficient for analysis and subsequent use.

In addition, since each array is fabricated *de novo*, the integrity of each array synthesized is suspect. Without being able to verify that the array has been fabricated with high fidelity, the absence of a signal at a particular address cannot be unambiguously interpreted. The absence of signal could be due to a failed synthesis or immobilization at that address, rather than a lack of biological activity.

Deposition methods suffer additional drawbacks, as well. Automatic deposition generally uses a robotic fluid delivery system. The robot moves to specific locations on a substrate, delivering a specified amount of fluid. The fluid is deposited onto the substrate by either a non-contact ejector (such as an ink jet nozzle) or a contact ejector (such as a pen, quill, or fiber) which actually touches the substrate surface to release the fluid. Ink jets, pens, and quills are adaptations of common devices, and each have reliability problems. Ink jets work fine when the fluid has been carefully optimized for the nozzle. However, when depositing many different fluids through the same nozzle, optimization of each fluid is impractical. Pens and quills are very useful for deposition onto a small number of substrates but are too slow for cost-effective production. While a fiber piston delivery system shows promise as a reliable means of fluid deposition, it requires an unwieldy number of fibers for a very large number of reaction sites.

Regardless of how the two-dimensional arrays are fabricated, assays employing them also suffer significant drawbacks. Because the entire array must be contacted with enough assay solution to completely cover the array, relatively large volumes, and hence quantities, of target analyze are required for each assay.

Thus, there remains a need in the art for libraries or arrays of immobilized compounds which can be easily and inexpensive prepared with a high degree of fidelity and which require very small quantities and volumes of target analytes in use. Accordingly, these are objects of the present invention.

4. SUMMARY OF THE INVENTION

These and other objects are furnished by the present invention, which in one aspect provides a linear array apparatus comprising a tube or channel, for example a capillary tube or etched channel, having disposed therein a plurality of individual beads aligned in a single-file linear array. Each bead has immobilized thereon a compound that

is potentially capable of binding with a target analyte of interest. The outer diameters of the beads are smaller than the inner lumen of the tube or channel such that the beads do not sealingly engage the inner lumen, thereby permitting assay solutions to flow through the channel or capillary and around the beads. Moreover, the outer diameters of the beads are large enough so that individual beads cannot pass by one another when disposed in the tube or channel. Thus, once dispensed in the tube or channel, the beads retain their linear alignment in use. The identities of the compounds immobilized on each bead are known such that the compounds are identifiable by their position within the linear array (linear spatial address). The linear array may optionally contain one or more marker beads that can be used as positional markers to aid the interpretation of results obtained with the linear arrays.

The linear array apparatus can be advantageously used in any currently available or later developed technologies and methods that employ spatially-addressable arrays of immobilized compounds. For example, the apparatus can be used to simultaneously detect the presence of multiple target analytes in a sample or to identify compounds which interact with a target analyte of interest, such as, for example, to identify a peptide or other compound which binds an antibody, enzyme or other receptor of interest. When the immobilized compounds are polynucleotides, the devices are particularly advantageous for use in fingerprinting, sequencing and other hybridization-based applications.

The linear arrays of the invention combine the advantageous features of conventional bead-based libraries and spatially-addressable arrays, and as a consequence provide significant advantages over both of these types of libraries. First, the beads used to construct the arrays can be prepared in bulk using conventional chemistries and stored for later use. As a consequence, the integrity of the synthesis of each member of the array can be verified prior to use by analyzing one or a few beads from the batch synthesis. Second, the average quantities of compounds immobilized on the beads can be determined. In applications where signal intensities are important, signals from different beads can therefore be normalized to account for differences in the amounts of compounds immobilized on the beads. This is particularly advantageous in applications involving nucleic acid hybridization, such a nucleic acid fingerprinting and SBH applications, as

signal intensities are related to the extent of hybridization between immobilized polynucleotides and target nucleic acids.

Third, because the linear arrays are constructed by simply dispensing pre-synthesized beads into a channel or capillary, customized arrays especially suited for particular applications can be conveniently and easily fabricated on an as needed basis. Beads having the desired compounds immobilized thereon are simply dispensed in a capillary or channel and the linear array is ready for use.

Lastly, owing to the micro dimensions of the linear arrays, assays can be performed using extremely small quantities and volumes of target analytes. Oftentimes, an entire array can be interrogated with microliter volumes of target analyte. Thus, the linear arrays of the invention are particularly advantageous for use in applications where only limited quantities and volumes of target analyte are available. Quite significantly, the linear arrays of the invention can be used in hybridization assays to fingerprint and/or sequence target nucleic acids isolated from biological samples without first having to amplify the target nucleic acid.

5. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a cross-sectional view of one embodiment of a linear array apparatus according to the invention;

FIG. 2 is a cross-sectional view of the linear array apparatus of FIG. 1 along line II-II;

FIG. 3 is a top view of another embodiment of a linear array apparatus according to the invention;

FIG. 3A is an alternative embodiment of the linear array of FIG. 3;

FIG. 4 is cross-sectional view of the linear array apparatus of FIG. 3 along line IV-IV;

FIG. 5 is the same view of the linear array apparatus of FIG. 3 depicted as FIG. 4, but illustrates an alternative cover plate;

FIG. 6 is an illustration of one mode of detection according to the invention;

FIG. 7 is an illustration of an alternative mode of detection according to the invention;

FIG. 8 is a top view of one embodiment of a microfunnel according to the invention;

FIG. 9 is a cross-sectional view of the microfunnel of FIG. 8 along line IX-IX;

FIG. 10 is a cross-sectional view of the microfunnel of FIG. 8 along line X-X;

FIG. 11 shows the results of a gene expression assay performed with a linear array of the invention; and

FIG. 12 shows the results of a format III SBH-type hybridization and ligation assay performed with a linear array of the invention.

6. DETAILED DESCRIPTION OF THE INVENTION

FIGS. 1-2 are various views of one embodiment of a linear array apparatus of the present invention. Referring to FIG. 1, linear array apparatus **10** comprises a tube **12** having an inner wall **13** and an outer wall **16**. Inner wall **13** defines an inner lumen **14** through which solutions containing a target analyte of interest (not shown) are flowed. Tube **12** has disposed therein a plurality of array beads **18** arranged in a linear array. Each bead **18** has immobilized thereon a compound **22**, optionally by way of a linker **24**, that is potentially capable of binding to, or otherwise interacting with, a target analyte of interest (not shown). The structure or identity of compound **22** immobilized on bead **18** is known such that the identities of the compounds which compose the linear array are defined by their linear positions within tube **12** (*i.e.*, by their linear spatial addresses).

As depicted in FIG. 1, each bead **18** may have a different compound immobilized thereon (illustrated by the filled circles, squares and triangles). However, as will be recognized by those having skill in the art, the individual beads composing the linear array need not be unique. Depending on the particular application, the linear arrays of the invention may contain several identical beads, and may even contain one or several replicate linear arrays arranged in the same tube.

Moreover, while in many instances each bead **18** will have a single compound immobilized thereon, each bead can have two, three, four or even more compounds immobilized thereon, depending upon the particular application. The immobilized compounds may be, but need not be members of the array. For example, a single bead can have immobilized thereon two different peptides whose binding characteristics with a

target analyte are of interest. Alternatively, a single bead can have immobilized thereon a single peptide whose binding characteristics with a target analyte are of interest and a "marker compound," such as a fluorophore, which can be used, *e.g.*, to mark the position of the bead in the linear array. Other arrangements of compounds which can be immobilized on a single bead **18** will depend upon the particular application and will be apparent to those of skill in the art.

In addition to beads **18**, tube **12** may also have disposed therein one or more marker beads **20**, which are typically used as positional markers in assays employing the linear arrays, but may also be used to space individual beads **18** apart from one another. Marker beads **20** have similar sizes and shapes as beads **18** (discussed *infra*), except that they typically will not have a compound **22** immobilized thereon. In order to provide their intended function of acting as positional markers in the linear array, marker beads **20** are detectable in some manner. For example, marker beads **20** may be detectable by their shape, by having a visible color or by way of some other detectable physical attribute. Alternatively, marker beads **20** can have immobilized thereon, typically by way of covalent attachment, a fluorophore or other label which produces a detectable signal when the linear array is scanned for signal following an assay. The label may be the same as, or different from, the label used to detect an interaction between immobilized compounds **22** and the target analyze. A wide variety of labels and modes of detection suitable for labelling the target analyte are discussed *infra*. Any of these labels can be used to label marker beads **20**.

The shape of beads **18** and marker beads **20** is not critical and may range from spherical, cylindrical, elliptical, cubical, etc. to irregularly shapes globules, although spherical beads are preferred. Regardless of their shapes, beads **18** and marker beads **20** must satisfy two important criteria. First, since it is desirable to flow assay solutions through tube **12** and around beads **18** and **20**, the beads should not sealingly engage the inner lumen **14** of tube **12**. This can be accomplished by ensuring that their largest outer dimension is smaller than the diameter of inner lumen **14** (*see* FIG. 2). In addition, since it is desirable to retain the linear spatial positions of beads **18** and **20** within tube **12**, the outer dimensions of beads **18** and **20** should be large enough so that the beads cannot pass by one another when disposed within tube **12**. For spherical beads **18** and **20** disposed

within a capillary tube having an inner lumen of diameter x, these criteria can be satisfied using beads having an outer diameter of less than x and greater than ½ x.

The ratio of the diameter of inner lumen **14** to the outer dimension of beads **18** and **20** is not critical. As will be discussed more thoroughly below, the size of beads used will depend, in part, upon the inner lumen diameter of tube **12**. Generally, spherical beads having an outer diameter in the range of 0.1 μm to 5000 μm are suitable, with outer diameters in the range of 1 μm to 100 μm being preferred. Of course, beads having larger or smaller outer diameters can also be used, depending on the choice of tube **12** and availability of the beads. Preferably, the largest outer dimension of beads **18** and **20** is only slightly smaller than the diameter of inner lumen **14**. However, the beads should not be so large that they cannot be easily dispensed into tube **12** or cause the linear array to develop significant back-pressures upon flowing assay solutions through the device. Maximizing the outer dimensions of the beads in this manner minimizes the volume of assay solution required to fill tube **12**. Choosing beads and tubes having dimensions suitable for specific applications will be apparent to those of skill in the art.

The shape of tube **12** will depend upon the particular application in which linear array apparatus **10** will be used. Typically tube **12** will be cylindrical, although other geometries or configurations can also be used. Also, as a particular advantage of the invention is the ability to use extremely small quantities and volumes of target analyte, tube **12** will typically be a capillary tube having an inner lumen diameter in the range of about 1 μm to 5000 μm or less, preferably in the range of about 1 μm to 150 μm, although tubes having larger or smaller inner lumen diameters could be used, depending on the amount of target analyte available and/or the availability of beads having the appropriate dimensions.

While the outer diameter of tube **12** is not critical, the tube should be sufficiently thick that it does not easily break during fabrication and use of the linear array. Preferably, the ratio of the outer and inner diameters of tube **12** will be in the range of 3 to 10, although thinner or thicker walled tubes **12** can be used.

The length of tube **12** will depend, in part, on the amount of target analyte available and the number of compounds composing the desired linear array. Depending upon the desired application, the linear arrays of the invention may comprise from as few

as two, to as many as thousands, tens of thousands, hundreds of thousands, or even more array members. As used herein, an "array member" is a bead **18** having immobilized thereon at least one compound **22** that is potentially capable of interacting with a target analyte of interest. As discussed *supra*, all of the array members may be, but need not be, unique. For example, the linear array of the invention may comprise 10000 beads, each having a unique peptide immobilized thereon. Alternatively, the linear array may comprise 10000 beads and only 2500 unique peptides. In this latter embodiment, the array would contain 4 replicate copies of each unique peptide. The complexity and the size of the array will depend upon the particular application, and will be apparent to those having skill in the art.

In theory, tube **12** can be long enough to contain an entire array. For example, a linear array comprising 1000 members utilizing 100 μm beads is only 10 cm long. In practice, however, the desired array may be divided amongst a plurality of linear array apparatuses. For example, if one desires to construct a linear array comprising the complete set of octanucleotides (4^8 = 65,536 unique octanucleotides) using the above-described 100 μm beads, one could use a single 6.55 meter long capillary tube having disposed therein 65,536 beads **18**, each of which has a unique octanucleotide immobilized thereon. Alternatively, one could use a plurality of capillary tubes, each of which has disposed therein a subset of the octanucleotide library. For example, one could use 64 capillaries (each 10 cm long), each of which has disposed therein 1024 beads having a unique octanucleotide immobilized thereon such that, *in toto,* the 64 capillaries compose the complete set of octanucleotides.

Thus, as those of skill in the art will appreciate, the length of the capillary used can be conveniently adjusted to suit the particular application. Generally, a length of tube **12** will be used such that the void volume of the linear array is in the range of about 2μl to 25 μl, preferably in the range of about 2 μl to 10 μl.

Tube **12** can be composed of virtually any material or mixture of materials that are compatible with the desired conditions of use. Preferably, tube **12** will not substantially deform, dissolve or melt under the assay conditions. Suitable materials include, but are not limited to, plastics such as acrylic, styrene-methyl methacrylate copolymers, ethylene/acrylic acid, acrylonitrile-butadiene-styrene (ABS), ABS/polycarbonate,

ABS/polysulfone, ABS/polyvinyl chloride, ethylene propylene, ethylene vinyl acetate (EVA), nylons (including nylon 6, nylon 6/6, nylon 6/6-6, nylon 6/9, nylon 6/10, nylon 6/12, nylon 11 and nylon 12), polycarylonitrile (PAN), polyacrylate, polycarbonate, polybutylene terephthalate (PBT), polyethylene terephthalate (PET), polyethylene (including low density, linear low density, high density, cross-linked and ultra-high molecular weight grades), polypropylene homopolymer, polypropylene copolymers, polystyrene (including general purpose and high impact grades), polytetrafluoroethylene (PTFE), fluorinated ethylene-propylene (FEP), ethylene-tetrafluoroethylene (ETFE), perfluoroalkoxyethylene (PFA), polyvinyl fluoride (PVF), polyvinylidene fluoride (PVDF), polychlorotrifluoroethylene (PCTFE), polyethylene-chlorotrifluoroethylene (ECTFE), polyvinyl alcohol (PVA), silicon styrene-acrylonitrile (SAN) and styrene maleic anhydride (SMA); metals; and glass. Preferably, tube **12** is transparent to the wavelengths of light used to illuminate and detect fluorophore labels commonly used in biological assays. A particularly preferred material is glass. Glass capillary tubes having suitable inner diameters are available commercially from, *e.g.*, Drummond Scientific Co., Hilgenberg GmbH, Richard Glass Co. and Friedrich & Dimmock Inc.. The inner wall **13** of tube **12** can be silanized or otherwise coated with materials or chemicals in order to decrease or prevent the target analyte of interest from non-specifically binding to the tube.

Beads **18** and **20** can be rough, smooth, porous or non-porous. However, as important aspects of the invention include the ability to minimize the quantities and volumes of target analyte necessary to perform an assay and the ability to detect very small quantities of target analyte, preferably at least beads **18**, and more preferably both beads **18** and beads marker **20**, are substantially non-porous. By substantially non-porous is meant that when assay solutions are flowed through the device, they will generally flow around beads **18** (and optionally marker beads **20**), rather than through beads **18**. While the surface of the beads may contain pores that, for example, increase their surface area (*e.g.*, controlled-pore glass), smooth beads are preferred. Beads that are smooth and non-porous provide two significant advantages. First, they decrease the void volume of the linear array device, thereby decreasing the volumes of analyte solution that must be used in assays employing the device. Second, they concentrate the immobilized compound on

the outside of the bead, where it can be more easily detected in assays employing detection labels whose signals do not readily pass through the bead material.

Beads **18** can be composed of virtually any material or mixture of materials suitable for immobilizing the desired compounds thereon. A material or mixture of materials should be selected such that beads **18** do not substantially dissolve, melt or otherwise deform under the conditions used to immobilize the compounds or under the desired assay conditions. In addition, the material or mixture of materials should not readily release the immobilized compound under conditions of use. Suitable materials include those listed above in connection with tube **12**. The actual choice of materials will depend upon, among other factors, the identity of the compound immobilized and the mode of immobilization, and will be apparent to those of skill in the art.

As will be discussed in more detail below in conjunction with the preparation of beads **18**, in embodiments where compound **22** is covalently attached to bead **18**, the bead is preferably composed of a material or mixture of materials that can be readily activated or derivatized with reactive groups suitable for effecting the covalent attachment. A wide variety of materials that can be activated with reactive groups suitable to effect covalent attachment of molecules are known in the art, and include, by way of example and not limitation, the plastics listed above in connection with tube **12** and glass. Due to its inert properties under most synthetic conditions and its ability to be easily activated with a plethora of reactive groups, glass is a preferred bead material in embodiments employing covalent attachment. Glass beads having suitable diameters are available commercially from, for example, Duke Scientific Co., Mo-Sci Co. and Potters Industries Inc.

It has been discovered that metal beads such as those composed of or coated with, for example, silver or gold, produce very low background signals. Thus, beads composed of or coated with metals such as silver or gold are also preferred. Silver or gold-coated beads having suitable dimensions for use in the linear arrays of the invention are commercially available from Potters Industries Inc.. Chemistries for covalently attaching biological and other molecules to such metal-coated beads, including peptides and polynucleotide, are known in the art (*see, e.g.*, Herne & Tarlov, 1997, J. Am. Chem. Soc. 119: 8916-8920 and the references cited therein).

A key feature of the linear arrays of the invention is their spatial addressability, *i.e.*, the compounds immobilized on beads **18** are identifiable by their position within the linear array, either relative to an end of tube **12**, a defined marking on tube **12**, one or more marker beads **20** or a terminal bead. To construct a linear array having such features, one end of tube **12** can be conveniently closed with a plug **26**. As illustrated in FIG. 1, plug **26** can sealingly engage inner lumen **14**. To permit assay solutions to flow through the device, plug **26** is preferably composed of porous material, such as glass wool or a glass frit. Alternatively, plug **26** may be a pin, wire or other implement having an outer diameter large enough to retain beads **18** and **20** and smaller than inner lumen **14**, thereby permitting the flow of assay solutions through the linear array device. Such a pin or wire can be fixedly attached to tube **12**, or can be held in place by external means.

Beads **18** are then dispensed into tube **12**. The identity or structure of the compound immobilized on each bead **18** is recorded as it is dispensed into tube **12**, conveniently with the aid of a computer. Beads **18** can be dispensed the tube **12** by hand, or with the aid of funnels and/or automated sorting and dispensing equipment suitable for use with capillary tubes and beads having the preferred sized discussed above. To aid reading of the device in an assay, marker beads **20** can optionally be dispensed into the device. Typically, marker beads **20** will be dispensed at regular intervals, such as every 5 or every 10 beads **18**. When dispensed in such a fashion, marker beads **20** provide internal reference markers from which the positions of particular beads **18** may be readily ascertained. Once all of the desired beads **18** and marker beads **20** have been dispensed into tube **12**, they can be conveniently kept in place by closing the other end of tube **12** with a plug **26**, as previously described.

For relatively small beads and capillaries, the beads may be conveniently dispensed into tube **12** with the aid of a microfunnel. Referring to FIG. 8, one embodiment of a microfunnel **100** that works particularly well with 100 μm beads and 150 μm i.d. capillaries comprises a substrate **102**, which preferably has at least one flat surface. Substrate **102** can be composed of any material or mixture of materials that are easily etched, machined or molded to include the features of the microfunnel, such as plastic, glass and metals. A preferred material is anodized aluminum.

Substrate **102** contains a channel **104** for receiving tube **12** and one or a plurality of reservoirs **106** for holding and dispensing the beads (not shown). Reservoirs **106** are connected to one another and to channel **104** *via* channel **108**. Referring to FIGS. 9 & 10, channel **108** meets channel **104** such that when tube **12** is disposed in channel **104**, channel **108** is aligned with the inner lumen **14** of tube **12**.

In use, tube **12** is positioned within channel **104** such that its inner lumen aligns with channel **108**. Beads (not shown) are dispensed into reservoirs **106**. Individual beads are then moved from reservoir **106** through channel **108** and into tube **12**. The beads are conveniently moved through channel **106** by hand, optionally with the aid of a tweezer or other implement and a microscope.

Microfunnel **100** can be conveniently prepared by etching, machining or molding, depending upon the choice of material(s) selected for substrate **102**. The dimensions of channels **104** and **108** and reservoirs **106** will depend upon, among other factors, the dimensions of the beads and tube **12**. Adjusting the dimensions to suit particular tubes **12** and beads is well within the capabilities of those of skill in the art.

FIG. 3 shows another embodiment of a linear array apparatus according to the invention. Referring to FIG. 3, linear array apparatus **10A** is similar to linear array apparatus **10**, with the exception that beads **18** are disposed within a channel **40** disposed within a solid substrate **42**. Solid substrate **42** is typically a flat, planar substrate such as a glass sheet, but may be composed of other materials, such as plastic or metal. Channel **40** may be coated with materials or chemicals that decrease or prevent the target analyte from non-specifically binding to the channel. Channel **40** may also be provided with a reflective coating or surface, which can act to amplify the signals produced from certain labels, *e.g.*, light-emitting labels.

Channel **40** can be etched or otherwise machined into the surface of an existing solid substrate **42**, or the solid substrate can be fabricated to contain channel **40**, such as by molding. The manner of making linear array apparatus **10A** will depend upon, among other factors, the material(s) composing substrate **42**, and will be apparent to those of skill in the art.

Like linear array apparatus **10**, the channel **40** of linear array apparatus **10A** can be from a few millimeters to a few microns, or even less, in width. A particular

advantage of linear array apparatus **10A** is that photolithography techniques can be used to etch channels **40** in silicon or glass wafer substrates **42** that have widths as small as a few microns, or even smaller. As depicted in FIG. 3A, which provides an alternative embodiment of linear array **10A**, the length of channel **40** can be equal to or shorter than the length of substrate **42**. In this former embodiment, beads **18** can be held within channel **40** with the aid of plugs **26**. Again, the actual length of channel **40** will depend upon the size of the desired array and the particular application. When large arrays are desired, a single solid substrate **42** can contain a plurality of channels **40**, as shown in FIGS. 3 and 3A.

The cross-sectional geometry of channel **40** is not critical. Thus, while FIGS. 4-5 (discussed *infra*) depict channels having a square cross-section, it will be appreciated that channels having other cross-sectional shapes, such as rectangular, semi-circular, semi-elliptical etc., can be used. As for linear array device **10**, beads **18** (and optionally marker beads **20**) should not sealingly engage channel **40**, and should be large enough so that they cannot pass by one another in use, thereby preserving their linear spatial arrangement.

Referring to FIGS. 4-5, linear array apparatus **10A** may optionally include a cover plate **44** which preferably sealingly engages solid substrate **42**. Cover plate **42** can be flat as depicted in FIG. 4, or may contain a channel **46** that mates with channel **40**, as depicted in FIG. 5. When mated channels **40** and **46** are used, the largest dimension of beads **18** should be smaller than the diameter of the mated channel so that the beads do not sealingly engage the mated channel, and large enough so that they retain their linear alignment in use.

Cover plate **44** can be constructed of virtually any material or mixture of materials that are inert to the desired assay conditions, as previously described for substrate **42**. To aid detection of light emitting and/or absorbing labels, cover plate **44** is preferably transparent. Preferred transparent materials include ion-fluorescence plastics and glass.

In another alternative embodiment, the linear array could comprise beads strung together on a wire, fiber, or other type of string. In this embodiment the entire string is placed within a capillary prior to assaying the array. Beads having holes with suitable diameters for stringing on a wire or other fiber can be manufactured with the use of a

laser. Like linear array **10**, smooth glass beads are preferred. The string should have a tensile strength sufficient to manipulate the array. Suitable materials include metals such as copper, aluminum, etc., nylons, gore tex® (Gore Company), teflon, fiberglass, etc.

Many of the various embodiments of the linear arrays of the invention are illustrated throughout the specification with specific reference to linear array apparatus **10**. It will be recognized, however, that this description is for illustrative purposes only, and that the various embodiments apply equally well to linear array apparatus **10A** and other alternative arrays described herein.

In general operation and use of linear array **10**, a solution containing a target analyte of interest is dispensed into tube **12**. The solution may be dispensed using any method known in the art for dispensing a fluid through a tube, such as pumping, aspirating, gravity flow, electrical pulsing, vacuum or suction, capillary action or electro-osmosis. Enough analyte solution is dispensed into tube **12** to ensure contact with all of the beads **18** disposed therein. Preferably, tube **12** is completely filled with the analyte solution. Beads **18** are contacted with the analyte solution under conditions that, and for a period of time sufficient to, promote an interaction, typically binding, between the compounds immobilized on beads **18** and the target analyte. In instances where excess target analyte interferes with the detection of the interaction, the analyte solution may be removed and the beads optionally washed prior to detection by flowing wash solution through the linear array. The interaction, if any, between the target analyte and the compounds immobilized on beads **18** is then analyzed.

In some instances, it may be desirable to provide mixing during the assay. This can be conveniently accomplished by placing the long axis of the linear array perpendicular to the ground and inverting the linear array several times, allowing the beads to settle under the force of gravity between each inversion. Of course, the ends of the linear array should be plugged so that the beads and assay fluid do not flow out of the device.

Alternatively, the assay solution can be mixed by continuously pumping it through the device for the duration of the assay, or pumping it back and forth through the device. In this mode, turbulance caused from the moving solution flowing past the beads mixes the solution. In yet another embodiment, the solution can be mixed with the aid of an

oscillating magnetic field. In this embodiment, magnetic beads are placed at the ends of the linear array (*i.e.*, the terminal beads in the array are magnetic beads) and an oscillating magnetic field is used to move the entire array back and forth. The oscillating magnetic field can be conveniently applied with a hand magnet by moving the magnet back and forth along the long axis of the linear arrray.

During certain assays, such as those involving hybridization of nucleic acids, it may be desirable to control the temperature of the linear array device during the course of the assay. This can be achieved using a variety of conventional means. For example, the device may be contacted with an appropriately controlled external heat source or placed within a heat block. Alternatively, the linear array device may be constructed from materials that act as heat conductors, such as anodized aluminum, copper, etc. This alternative embodiment is particularly suited to linear array apparatus **10A**, as the substrate material can be a conductor such as anodized aluminum and contacted with an appropriately controlled external heat source. In this mode, the linear array acts as a heat sink to control the temperature of the assay solution.

The method by which the interaction is analyzed will depend upon the particular assay. For example, where the immobilized compound and target analyte each constitute one member of a binding pair of molecules (for example, a ligand and its receptor or two complementary polynucleotides), the interaction can be conveniently analyzed by labeling one member of the binding pair, typically the target analyte, with a moiety that produces a detectable signal upon binding. Only those beads in the linear array where binding has taken place will produce a detectable signal.

Any label capable of producing a detectable signal can be used. Such labels include, but are not limited to, radioisotopes, chromophores, phosphorophores, fluorophores, lumophores, chemiluminescent moieties, etc. The label may also be a compound capable of producing a detectable signal, such as an enzyme capable of catalyzing, *e.g.*, a light-emitting reaction or a colorimetric reaction (*e.g.*, alkaline phosphatase, glucose oxidose, horseradish peroxidase, urease, luciferase, galactosidase, etc.). Preferably, the label is a moiety capable of absorbing or emitting light, such as a chromophore or a fluorophore.

Alternatively, both the immobilized compound and target analyte are unlabeled and their interaction is indirectly analyzed with a reporter moiety that specifically detects the interaction. For example, binding between an immobilized antigen and a first antibody (or *visa versa*) could be analyzed with a labeled second antibody specific for the antigen-first antibody complex. For applications involving hybridization between polynucleotides, the presence of hybrids could be detected by intercalating dyes, such as ethidium bromide, which are specific for double-stranded polynucleotides.

Those of skill in the art will recognize that the above-described modes of detecting an interaction between an immobilized compound and a target analyte are merely illustrative. Other methods of detecting myriad types of interactions between compounds and analytes are well known in the art and can be readily used or adapted for use with the linear arrays of the present invention.

The binding of target analytes to beads **18** of the linear arrays of the invention can be facilitated by, *e.g.*, electrophoretic and/or mechanical pumping of the target analyte solution through the apparatus. In the case of electrophoresis, the inner lumen diameter of tube **12** is typically less than about 1-2 millimeters, preferably less than about 500 microns. For example, a capillary tube having an internal lumen diameter of about 100 microns and a length of about 50 millimeters can be used. For applications involving hybridization of polynucleotides, the use of capillaries having a high surface area to volume ratio facilitates more rapid hybridization kinetics, in part because any target analyte polynucleotide that passes through the capillary is in close proximity to the polynucleotides immobilized on the beads. In addition, capillaries having a high surface area to volume ratio have a high capacity for dissipating heat, thereby permitting the use of high voltages, which can accelerate the assays. Methods of carrying out capillary electrophoresis are well-known in the art, and are described, *e.g.*, in Novotny *et al.*, 1990, Electrophoresis 11:735-749. Any of several standard capillary electrophoresis systems can be used in conjunction with the linear arrays of the invention. For example, the Beckman P/ACE System 2050 (Beckman Instruments, Columbia, MD) can be used. In addition, systems that facilitate simultaneous processing of several capillary tubes can be used, such as those described in U.S. Patent No. 5,324,401.

Other electrophoretic methods can also be used in conjunction with the linear arrays of the invention. For example, electrophoresis can be carried out with etched linear arrays **10A**. In this method, each end of channel **40** is in contact with electrodes across which a voltage is applied to electrophoretically move the target analyte through channel **40**.

Once a linear array according to the invention has been used in an assay, emission, absorbance or another detectable signal produced by specific beads **18** where an interaction with the target analyte has taken place can be detected using any of several standard methods, depending upon the particular nature of the label. For example, chromophore labels can be detected by measuring the absorbance of light at a specific wavelength with a spectrophotometer, while light generated by a chemiluminescent label can be detected using a luminometer or a charge-coupled device (CCD). Fluorescence generated from a fluorophore label that has been excited at an appropriate wavelength (*e.g.*, with a standard mercury light source or a laser beam) can be detected with a confocal microscope, spectrophotometer or a CCD device. Radioactive labels can be detected by exposure to film, or by using a CCD device. Colorimetric labels can be detected by observing the beads with the eye, optionally with the aid of a microscope.

To detect multiple beads, the linear array can be placed adjacent to the detector. For example, when radioactive labels are used, the linear array can be placed upon a piece of film. The linear array can contain either a labeled marker bead or a radioactive marking at one end of the tube that acts as a position reference marker. The developed film will contain signals only at those linear positions corresponding to the reference markers and beads where binding took place. Since the identities of the compounds immobilized on individual beads within the linear array are identifiable by their spatial addresses, the identities of compounds which produced binding interactions can be readily obtained from the positions of the signals produced on the film. Where quantification of label is desired, the film can be analyzed with a densitometer, such as those typically used in conjunction with gel electrophoresis.

When fluorescent labels are used, multiple beads can be detected using a scanning confocal microscope, a scanning fiber optic device or a CCD device can be placed adjacent to the capillary, but perpendicular to the excitation beam. The excitation beam

can be part of the scanning device. For example, in a scanning fiber optic device an inner fiber can carry the laser light while surrounding fibers carry the fluorescent light back to an imaging system. In a particularly convenient mode of fluorescence detection for use with linear arrays **10**, the excitation laser beam can be directed through one end of tube **12**, either through the inner lumen **14**, whereby the inside of the tube acts as a light tunnel, or through the external wall **16**, which then acts as a waveguide. In this latter case, only fluorophore labels very close to the inner wall **13** of the array would get excited through a well-known process called surface plasmon resonance. As will be apparent to those of skill in the art, appropriate filters to remove scattered or reflected light can be used with a detector device, such as a CCD device or a photomultiplier tube.

FIGS. 6-7 show the various detection geometries that can be used with the linear arrays of the invention. Referring to FIG. 6, detector **50** can be placed perpendicular to the long axis of linear array **10** and the linear array translated along its long axis (depicted as the *y*-axis) such that individual beads **18** and/or marker **20** pass sequentially by stationary detector **50**. In FIG. 6, labeled beads are shaded and unlabeled beads are unshaded. In this embodiment, linear array **10** is placed on a translatable surface, for example, an actuated *xy* translation table, whose translation is preferably under the control of a computer. Alternatively, detector **50** can be translated along the long axis of stationary linear array **10**. In yet another mode, a linear CCD device spanning the entire length of linear array **10** can be used.

In all instances, an imaging device can be used in conjunction with the detector to produce a bar code-like image **52** of the assayed linear array. Where signal intensities are important, a photon counter can be used to quantify the emission signals and the intensity information included in the image. The absolute intensities can be normalized based upon the quantities of compounds immobilized on the beads.

In yet another embodiment, which is not illustrated, the positions of the linear array and detector are fixed, and the individual beads are transported along the tube such that they move past the stationary detector. The beads can be transported along the tube *via* pumping, with the aid of pressurized air flow, or with the aid of a physical device such as a pin. Whether labeled or unlabeled, beads that pass by the detector are counted so that the observed signals can be correlated with specific beads in the linear array.

In yet another alternative embodiment, depicted in FIG. 7, detector **50** is positioned parallel to the long axis of linear array **10** and the signals from a two- or three-dimensional stack of linear arrays **10** detected. In this embodiment, the terminal bead **18** from each linear array **10** of the stack is analyzed for signal. In FIG. 7, labeled beads are shaded, unlabeled beads are unshaded. As with the other modes of detection, detector **50** can be translated along the stationary stack in a direction perpendicular to the long axes of the linear arrays, or the stack can be moved passed stationary detector **50**. Alternatively, the entire stack can be placed on a CCD device, with the long axes of the linear arrays perpendicular to the CCD, such that the entire cross-sectional area of the stack is detected simultaneously. After the terminal beads of each linear array in the stack have been scanned for signal, they are removed from their respective linear arrays, typically by transporting the beads through the tube as previously described, and the next beads in the linear arrays are scanned for signal.

Detecting in this mode produces a series of two-dimensional images **54**. Each two-dimensional image corresponds to a particular bead position. For example, nine linear arrays that each contain 10 beads arranged in a 3x3 stack will yield 10 3x3 two-dimensional images. By keeping track of which beads in the arrays produced a particular two-dimensional image, the spatial-addressability of the linear arrays is preserved. To aid the interpretation of the various two-dimensional images, the stack can be constructed so that every corner bead is a marker bead that bears a label (depicted in FIG. 7). Each two-dimensional image **54** will then have signals that define its corners.

Appropriate systems integrating signal induction (*e.g.*, lasers) and detection (*e.g.*, CCD devices) devices are well-known in the art. *See, e.g.*, U.S. Patent No. 5,069,769 and U.S. Patent No. 5,208,466. A system employing a confocal microscope and a computer actuated *xy* translation table suitable for use in the arrangement depicted in FIG. 6 is described in U.S. Patent No. 5,744,305, particularly at Cols. 29-32.

Those of skill in the art will recognize that the linear arrays of the invention can be used in virtually any assay where detecting interactions between compounds and analytes is desired. For example, the linear arrays can be conveniently used to screen for and identify compounds which bind a receptor of interest, such as peptides which bind an antibody, organic compounds which bind an enzyme or receptor, or complementary

polynucleotides which bind (hybridize to) one another. However, the arrays of the invention are not limited to applications in which the immobilized compounds and target analyte bind another. The arrays of the invention can also be used to screen for and identify compounds which catalyze chemical reactions, such as antibodies capable of catalyzing certain reactions, and to screen for and identify compounds which give rise to detectable biological signals, such as compounds which agonize a receptor of interest. The only requirement is that the interaction between the immobilized compound and target analyte give rise to a spatially-addressable detectable signal. Thus, the linear arrays of the invention are useful in any applications that take advantage of arrays or libraries of immobilized compounds, such as the myriad solid-phase combinatorial library assay methodologies described in the art. For a brief review of the various assays for which the linear arrays of the invention can be readily adapted, *see* Gallop *et al.*, 1994, J. Med. Chem. 37:1233-1251; Gordon *et al.*, 1994, J. Med. Chem. 37:1385-1401; Jung, 1992, Agnew Chem. Pat. Ed. 31:367-386; Thompson & Ellman, 1996, Chem Rev. 96: 555-600; and the references cited in all of the above.

Based on the above, those of skill in the art will recognize that the compounds immobilized on beads **18** can be virtually any types of compounds, ranging from organic compounds such as potential drug candidates, polymers and small molecule inhibitors, agonists and/or antagonists, to biological compounds such as polypeptides, polynucleotides, polycarbohydrates, lectins, proteins, enzymes, antibodies, receptors, nucleic acids, etc. The only requirement is that the compounds be capable of being immobilized on a bead.

The linear arrays of the invention are particularly useful for applications involving hybridization of nucleic acids, especially those applications involving high density arrays of immobilized polynucleotides, including, for example, nucleic acid fingerprinting, *de novo* sequencing by hybridization (SBH) and detection of polymorphisms. In these applications, the conventional two-dimensional arrays of immobilized polynucleotides are conveniently and advantageously replaced with the linear arrays of the invention. For a review of the various array-based hybridization assays in which the linear arrays of the invention find use, *see* U.S. Patent No. 5,202,231; U.S. Patent No. 5,525,464; WO 98/31836, and the references cited in all of the above.

As the linear arrays of the invention are particularly well suited for use in SBH and other nucleic acid-based hybridization assays, in a preferred embodiment, the compounds immobilized on beads **18** are polynucleotides. Typically, the polynucleotides will be of a strandedness and length suitable for use in format II and format III SBH and related applications. Thus, the polynucleotides will generally be single-stranded and be composed of between about 4 to 30, typically about 6 to 20, and usually about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 nucleotides. (Polynucleotides of these lengths are commonly referred to as oligonucleotide probes).

However, it will be recognized that the linear arrays of the invention are equally well suited for use with format I SBH and related applications, where an immobilized target nucleic acid is interrogated with solution-phase oligonucleotide probes. Accordingly, the polynucleotide immobilized on bead **18** can be any number of nucleotides in length, from as few as 4 to as many as hundreds, or even more, and can be either single- or double-stranded, depending on the particular application.

The polynucleotides composing the linear array can be of any desired sequence. In a preferred embodiment, they can comprise all possible polynucleotides of a given length k, which would result in a linear array of 4^k unique beads. For all polynucleotides of, for example, 6 bases in length, the sequences would comprise a linear array with 4096 unique beads. Alternatively, the polynucleotides can make up the "universal set" for sequencing a nucleic acid, as described in WO 98/31836, particularly pages 27-29.

In an alternative embodiment, the immobilized polynucleotides may correspond to particular mutations that are to be identified in a known target nucleic acid sequence. For example, if a particular target nucleic acid is known to contain an unidentified mutation at a particular position, then the mutated position can be identified with a linear array of eight polynucleotides: three corresponding to the three possible base substitutions at that position; one corresponding to the deletion of the base at that position; and four corresponding to the insertion of the four possible bases at that position. Alternatively, for a known gene that may contain any of several possible identified mutations, the linear array can comprise immobilized polynucleotides corresponding to the different possible mutations. This embodiment is particularly useful for genes like oncogenes and tumor suppressors, which frequently have a variety of known mutations in different positions.

Using linear arrays facilitates determining whether or not these genes contain mutations by allowing simultaneous screening with polynucleotides corresponding to each of these different positions.

In another alternative embodiment, each bead of the linear array can have immobilized thereon a mixture of polynucleotides of different sequences. These mixtures may comprise degenerate polynucleotides of the structure $N_xB_yN_z$, wherein N represents any of the four bases and varies for the polynucleotides in a given mixtures, B represents any of the four bases but is the same for each of the polynucleotides in a given mixture, and x, y, and z are integers. Typically, x and z are independently integers between 0 and 5 and y is an integer between 4 and 20. The number of known bases B_y defines the "information content" of the polynucleotide, since the degenerate ends do not contribute to the information content of the probes. Linear arrays comprising such mixtures of immobilized polynucleotides are useful in, for example, sequencing by hybridization. Hybridization discrimination of mismatches in these degenerate probe mixtures refers only to the length of the informational content, not the full physical length.

Alternatively, each bead in the linear array can have immobilized thereon mixtures of polynucleotides that correspond to different regions of a known nucleic acid; these regions may be overlapping, adjacent, or nonadjacent. Linear arrays comprising these types of mixtures are useful in, for example, identifying specific nucleic acids, including those from particular pathogens or other organisms. Both types of mixtures are discussed in WO 98/31836, particularly at pages 123-128.

Yet another useful mixture of probes that can be immobilized on a single bead is described in attorney docket no. 9598-064-999, filed January 6, 1999, incorporated herein by reference. Linear arrays utilizing these mixtures of probes are particularly suited for SBH applications.

The polynucleotide may be composed entirely of deoxyribonucleotides, entirely of ribonucleotides, or may be composed of mixtures of deoxy- and ribonucleotides. However, due to their stability to RNases and high temperatures, as well as their ease of synthesis, polynucleotides composed entirely of deoxyribonucleotides are preferred.

The polynucleotide may be composed of all natural or all synthetic nucleotide bases, or a combination of both. While in most instances the polynucleotide will be

composed entirely of the natural bases (A, C, G, T or U), in certain circumstances the use of synthetic bases may be preferred. Common synthetic bases of which the polynucleotide may be composed include 3-methlyuracil, 5,6-dihydrouracil, 4-thiouracil, 5-bromouracil, 5-thorouracil, 5-iodouracil, 6-dimethyl amino purine, 6-methyl amino purine, 2-amino purine, 2,6-diamino purine, 6-amino-8-bromo purine, inosine, 5-methyl cytosine and 7-deaza quanosine. Additional non-limiting examples of synthetic bases of which the polynucleotide can be composed can be found in Fasman, CRC PRACTICAL HANDBOOK OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, 1985, pp. 385-392.

Moreover, while the backbone of the polynucleotide will typically be composed entirely of "native" phosphodiester linkages, it may contain one or more modified linkages. such as one or more phosphorothioate, phosphorodithioate, phosphoramidate or other modified linkages. As a specific example, the polynucleotide may be a peptide nucleic acid (PNA), which contains amide interlinkages. Additional examples of modified bases and backbones that can be used in conjunction with the invention, as well as methods for their synthesis can be found, for example, in Uhlman & Peyman, 1990, Chemical Review 90(4):544-584; Goodchild, 1990, Bioconjugate Chem. 1(3):165-186; Egholm *et al.*, 1992, J. Am. Chem. Soc. 114:1895-1897; Gryaznov *et al.*, J. Am. Chem. Soc. 116:3143-3144, as well as the references cited in all of the above.

While the polynucleotide will often be a contiguous stretch of nucleotides, it need not be. Stretches of nucleotides can be interrupted by one or more linker molecules that do not participate in sequence-specific base pairing interactions with a target nucleic acid. The linker molecules may be flexible, semi-rigid or rigid, depending on the desired application. A variety of linker molecules useful for spacing one molecule from another or from a solid surface have been described in the art (and are described more thoroughly *infra*); all of these linker molecules can be used to space regions of the polynucleotide from one another. In a preferred embodiment of this aspect of the invention, the linker moiety is from one to ten, preferably two to six, alkylene glycol moieties, preferably ethylene glycol moieties, in length.

The polynucleotide to be immobilized can be isolated from biological samples, generated by PCR reactions or other template-specific reactions, or made synthetically. Methods for isolating polynucleotides from biological samples and/or PCR reactions are

well-known in the art, as are methods for synthesizing and purifying synthetic polynucleotides. Polynucleotides isolated from biological samples and/or PCR reactions may, depending on the desired mode of immobilization, require modification at the 3'- or 5'-terminus, or at one or more bases, as will be discussed more thoroughly below. Moreover, since the immobilized polynucleotide must typically be capable of hybridizing to another target nucleic acid, if not already single stranded, it should preferably be rendered single stranded, either before or after immobilization on the bead.

Depending on the identity of the compound to be immobilized and the bead material, the compound can be immobilized by virtually any means known to be effective for immobilizing the particular type of compound on the particular type of bead material. For example, the compound can be immobilized *via* absorption, adsorption, ionic attraction or covalent attachment. The immobilization may also be mediated by way of pairs of specific binding molecules, such as biotin and avidin or streptavidin. Methods for immobilizing a variety of compounds on a variety of materials are known in the art. Any of these art-known methods can be used in conjunction with the invention.

For adsorption or absorption, bead **18** can be conveniently prepared by contacting the bead with the compound to be immobilized for a time period sufficient for the compound to adsorb or absorb onto the bead. Following optional wash steps, the bead is then dried. When the compound is a polynucleotide, the various methods described in the dot-blot or other nucleic acid blotting arts for immobilizing nucleic acids onto nitrocellulose or nylon filters can be conveniently adapted for use in the present invention.

For immobilization by ionic attraction, if not inherently charged, the bead is first activated or derivatized with charged groups prior to contacting it with the compound to be immobilized, which is either inherently oppositely charged or has been modified to be oppositely charged.

For immobilization mediated by way of specific binding pairs. the bead is first derivatized and/or coated with one member of the specific binding pair, such as avidin or streptavidin, and the derivatized bead is then contacted with a compound which is linked to the other member of the specific binding pair, such as biotin. Methods for derivatizing or coating a variety of materials with binding molecules such as avidin or streptavidin, as well as methods for linking myriad types of compounds to binding molecules such as

biotin are well known in the art. For polynucleotides, biotin can be conveniently incorporated into the polynucleotide at either a terminal and/or internal base, or at one or both of its 5'- and 3'- termini using commercially available chemical synthesis or biological synthesis reagents.

In a preferred embodiment of the invention, the compound is covalently attached to the bead, optionally be way of one or more linking moieties. Unless the bead inherently contains reactive functional groups capable of forming a covalent linkage with the compound, it must first be activated or derivatized with such reactive groups. Typical reactive groups useful for effecting covalent attachment of compounds to beads include hydroxyl, sulfonyl, amino, cyanate, isocyanate, thiocyanate, isothiocyanate, epoxy and carboxyl groups, although other reactive groups as will be apparent to those of skill in the art may also be used.

A variety of techniques for activating myriad types of bead materials with reactive groups suitable for covalently attaching compounds thereto, particularly biological molecules such as polypeptides, proteins, polynucleotides and nucleic acids, are known in the art and include, for example, chemical activation, corona discharge activation, flame treatment activation, gas plasma activation and plasma enhanced chemical vapor deposition. Any of these techniques can be used to activate the bead with reactive groups. For a review of the many techniques that can be used to activate or derivatize the bead, *see* WILEY ENCYCLOPEDIA OF PACKAGING TECHNOLOGY, 2d Ed., Brody & Marsh, Ed., "Surface Treatment," pp. 867-874, John Wiley & Sons, 1997, and the references cited therein. Chemical methods suitable for generating amino groups on preferred glass beads are described in Atkinson & Smith, "Solid Phase Synthesis of Oligodeoxyribonucleotides by the Phosphite Triester Method," *In*: OLIGONUCLEOTIDE SYNTHESIS: A PRACTICAL APPROACH, M J Gait, Ed., 1985, IRL Press, Oxford, particularly at pp. 45-49 (and the references cited therein); chemical methods suitable for generating hydroxyl groups on preferred glass beads are described in Maskos *et al.*, 1992, Nucl. Acids Res. 20(7):1679-1684 (and the references cited therein); chemical methods suitable for generating functional groups on bead materials such as polystyrene, polyamides and grafted polystyrenes are described in Lloyd-Williams *et al.*, 1997, CHEMICAL APPROACHES TO THE SYNTHESIS OF PEPTIDES AND PROTEINS, Chapter 2, CRC Press, Boca Raton, FL (and

the references cited therein). Additional methods are well known, and will be apparent to those of skill in the art.

Depending on the nature of the compound to be immobilized, it can be covalently immobilized on an activated bead following synthesis and/or isolation, or, where suitable chemistries are known, it may be synthesized *in situ* directly on the bead. For example, a purified polypeptide may be covalently immobilized on an amino-activated bead, conveniently by way of its carboxy terminus or a carboxyl-containing side chain residue. Alternatively, the polypeptide can be synthesized *in situ* directly on an amino-activated bead using conventional solid-phase peptide chemistries and reagents (see CHEMICAL APPROACHES TO THE SYNTHESIS OF PEPTIDES AND PROTEINS, Lloyd-Williams *et al.*, Eds., CRC Press, Boca Raton, FL, 1997 and the references cited therein). Similarly, a purified polynucleotide bearing an appropriate reactive group at one or more of its bases or termini can be covalently immobilized on an isothiocyanate- or carboxy-activated bead, or alternatively, the polynucleotide can be synthesized *in situ* directly on a hydroxyl-activated bead using conventional oligonucleotide synthesis chemistries and reagents (see OLIGONUCLEOTIDE SYNTHESIS: A PRACTICAL APPROACH, 1985, *supra*, and the references cited therein). Other types of compounds which can be conveniently synthesized by solid phase methods can also be synthesized *in situ* directly on a bead. Non-limiting examples of compounds which can be synthesized *in situ* include Passerini and Ugi condensation products (WO 95/02566), peptoids (Simon *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:9367-9371), non-peptide non-oligomeric compounds (Dewitt *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:6909-6913) and 1,4 benzodiazepines and derivatives (Bunin *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712); Bunin & Ellman, 1992, J. Am. Chem. Soc. 114:10997-10998).

Multiple compounds may be conveniently synthesized in parallel using, for example, the "tea bag" approach of Houghten, 1985, Proc. Natl. Acad. Sci. USA 82: 5131-5135 (see also U.S. Patent No. 4,631,211), the automated methods described by Zuckerman *et al.*, 1992, Int. J. Pept. Protein Res. 40: 497-506 or the split synthesis described by Furka *et al.*, 1991, Int. J. Pept. Protein Res. 37: 487-493 (see also, Sebestyen *et al.*, 1993, Siorg. Med. Chem. Lett. 3: 413-418; Lam *et al.*, 1991, Nature 354: 82-84 and Houghten *et al.*, 1991, Nature 354: 84-86).

Those of skill in the art will recognize that when using *in situ* chemical synthesis, the covalent bond formed between the immobilized compound and the bead must be substantially stable to the synthesis and deprotection conditions used so as to avoid loss of the compound during synthesis and/or deprotection. For polynucleotides, one such stable bond is the phosphodiester bond, which connects the various nucleotides in a polynucleotide, and which can be conveniently formed using well-known chemistries (*see, e.g.*, OLIGONUCLEOTIDE SYNTHESIS: A PRACTICAL APPROACH, 1985, *supra*). Other stable bonds suitable for use with hydroxyl-activated beads include phosphorothiate, phosphoramidite, or other modified nucleic acid interlinkages. For beads activated with amino groups, the bond could be a phosphoramidate, amide or peptide bond. For beads activated with epoxy functional groups, a stable C-N bond could be formed. Suitable reagents and conditions for forming such stable bonds are well known in the art.

In one particularly convenient embodiment, a polynucleotide is immobilized by *in situ* synthesis on a hydroxyl-activated bead using commercially available phosphoramidite synthesis reagents and standard oligonucleotide synthesis chemistries. In this mode, the polynucleotide is covalently attached to the activated bead by way of a phosphodiester linkage. The density of polynucleotide covalently immobilized on the bead can be conveniently controlled by adding an amount of the first synthon (*e.g.*, N-protected 5'-O-dimethoxytrityl-2'-deoxyribonucleotide-3'-O-phosphoramidite) sufficient to provide the desired number of synthesis groups on the bead, and capping any unreacted hydroxyl groups on the bead with a capping reagent (*e.g.*, 1,4-diaminopyridine; DMAP). After the excess hydroxyls have been capped, the trityl group protecting the 5'-hydroxyl can be removed and synthesis of the polynucleotide carried out using standard techniques. Following synthesis, the polynucleotide is deprotected using conventional methods.

In an alternative embodiment, the polynucleotide is covalently attached to the activated bead through a post-synthesis or post-isolation conjugation reaction. In this embodiment, a synthesized or isolated polynucleotide which is modified at its 3'-terminus, 5'-terminus and/or at one of its bases with a reactive functional group (*e.g.* epoxy, sulfonyl, amino, isothiocyanato or carboxyl) is conjugated to an activated bead *via* a condensation reaction, thereby forming a covalent linkage. Again, substantially stabile (*i.e.*, non-labile) covalent linkages such as amide, phosphodiester and phosphoramidate

linkages are preferred. Synthesis supports and synthesis reagents useful for modifying the 3'- and/or 5'-terminus of synthetic polynucleotides, or for incorporating a base modified with a reactive group into a synthetic polynucleotide, are well-known in the art and are commercially available. Preferred methods for covalently attaching amino-modified polynucleotides to glass heads activated with isothiocyanate groups is provided in the example.

For example, methods for synthesizing 5'-modified oligonucleotides are described in Agarwal et al., 1986, Nucl. Acids Res. 14:6227-6245 and Connelly, 1987, Nucl. Acids Res. 15:3131-3139. Commercially available products for synthesizing 5'-amino modified oligonucleotides include the N-TFA-C6-AminoModifer™, N-MMT-C6-AminoModifer™ and N-MMT-C12-AminoModifier™ reagents available from Clontech Laboratories, Inc., Palo Alto, California.

Methods for synthesizing 3'-modified oligonucleotides are described in Nelson et al., 1989, Nucl. Acids Res. 17:7179-7186 and Nelson et al., 1989, Nucl. Acids Res. 17:7187-7194. Commercial products for synthesizing 3'-modified oligonucleotides include the 3'-Amino-ON™ controlled pore glass and Amino Modifier II™ reagents available from Clontech Laboratories, Inc., Palo Alto, California.

Other methods for modifying the 3' and/or 5' termini of oligonucleotides, as well as for synthesizing oligonucleotides containing appropriately modified bases are provided in Goodchild, 1990, Bioconjugate Chem. 1:165-186, and the references cited therein. Chemistries for attaching such modified oligonucleotides to materials activated with appropriate reactive groups are well-known in the art (*see, e.g.*, Ghosh & Musso, 1987, Nucl. Acids Res. 15:5353-5372; Lund *et al.*, 1988, Nucl. Acids Res. 16:10861-10880; Rasmussen *et al.*, 1991, Anal. Chem. 198:138-142; Kato & Ikada, 1996, Biotechnology and Bioengineering 51:581-590; Timofeev *et al.*, 1996, Nucl. Acids Res. 24:3142-3148; O'Donnell *et al.*, 1997, Anal. Chem. 69:2438-2443).

Methods and reagents for modifying the ends of polynucleotides isolated from biological samples and/or for incorporating bases modified with reactive groups into nascent polynucleotides are also well-known and commercially available. For example, an isolated polynucleotide can be phosphorylated at its 5'-terminus with phosphorokinase and this phosphorylated polynucleotide covalently attached to an amino-activated bead

through a phosphoramidate or phosphodiester linkage. Other methods will be apparent to those of skill in the art. A preferred method for activating glass beads with isothiocyanate groups suitable for covalently attaching amino-modified polynucleotides is provided in the examples.

Whether synthesized directly on the activated bead or immobilized on the activated bead after synthesis or isolation, the compound can be optionally spaced away from the bead by way of one or more linkers. As will be appreciated by those having skill in the art, such linkers will be at least bifunctional, *i.e.*, they will have one functional group or moiety capable of forming a linkage with the activated bead and another functional group or moiety capable of forming a linkage with another linker molecule or the compound to be immobilized. The linkers may be long or short, flexible or rigid. charged or uncharged, hydrophobic or hydrophilic, depending on the particular application.

In certain circumstances, such linkers can be used to "convert" one functional group into another. For example, an amino-activated bead can be converted into a hydroxyl-activated bead by reaction with, for example, 3-hydroxy-propionic acid. In this way, bead materials which cannot be readily activated with a specified reactive functional group can be conveniently converted into a an appropriately activated bead. Chemistries and reagents suitable for "converting" such reactive groups are well-known, and will be apparent to those having skill in the art.

Linkers can also be used, where necessary, to increase or "amplify" the number of reactive groups on the activated bead. For this embodiment, the linker will have three or more functional groups. Following attachment to the activated bead by way of one of the functional groups, the remaining two or more groups are available for attachment of the compound. Amplifying the number of functional groups on the activated bead in this manner is particularly convenient when the activated bead contains relatively few reactive groups.

Reagents for amplifying the number of reactive groups are well-known and will be apparent to those of skill in the art. A particularly convenient class of amplifying reagents are the multifunctional epoxides sold under the trade name DENACOL™ (Nagassi Kasei Kogyo K.K.). These epoxides contain as many as four, five, or even

more epoxy groups, and can be used to amplify beads activated with reactive groups that react with epoxides, including, for example, hydroxyl, amino and sulfonyl activated fibers. The resulting epoxy-activated beads can be conveniently converted to a hydroxyl-activated bead, a carboxy-activated bead, or other activated bead by well-known methods.

Linkers suitable for spacing biological or other molecules, including polypeptides and polynucleotides, from solid surfaces are well-known in the art, and include, by way of example and not limitation, polypeptides such as polyproline or polyalanine, saturated or unsaturated bifunctional hydrocarbons such as 1-amino-hexanoic acid and polymers such as polyethylene glycol, etc. For polynucleotide compounds, a particularly preferred linker is polyethylene glycol (MW 100 to 1000). 1,4-Dimethoxytrityl-polyethylene glycol phosphoramidites useful for forming phosphodiester linkages with hydroxyl groups of hydroxyl-activated beads, as well as methods for their use in nucleic acid synthesis on solid substrates, are described, for example in Zhang *et al.*, 1991, Nucl. Acids Res. 19:3929-3933 and Durand *et al.*, 1990, Nucl. Acids Res. 18:6353-6359. Other useful linkers are commercially available.

Significant advantages of the linear arrays of the invention include the ability to verify the integrity of the synthesis or attachment chemistry prior to use and to quantify the amount of compounds immobilized on the beads. To verify the synthesis integrity and/or quantify the amount of immobilized compound, the compound from one or a few beads can be cleaved from the bead and analyzed using standard analytical and/or quantitative techniques. For example, the cleaved compound can be analyzed by HPLC, spectrophotometry, mass spectrometry, etc. The actual techniques used will depend upon the identity of the immobilized compound and the nature of the linkage, and will be apparent to those of skill in the art. The average amount of compound immobilized on a bead can be determined by dividing the amount of compound cleaved from a few representative beads by the total number of beads from which the compound was cleaved.

For use in a typical hybridization-based assay where a target nucleic acid is interrogated with a linear array of immobilized polynucleotide probes, hybridization solution containing the target nucleic acid is flowed through the device as previously described. The target nucleic acid in the hybridization solution may be labeled or

unlabeled, depending on the particular assay (for example, in format II SBH, the target nucleic acid is labeled; in format III SBH the target nucleic acid is unlabeled). Moreover, the hybridization solution may optionally contain labeled oligonucleotide probes of known sequence and DNA ligane. Labeled probes of different sequ3nces should be labeled with different, distinguishable labels.

The hybridization solution is contacted with the array under conditions which allow discrimination between perfectly complimentary hybrids and hybrids containing one or more mismatches in the information content of the immobilized polynucleotide probes. The actual hybridization conditions used will depend upon, among other factors, the G+C content of the sequence of interest and the lengths of the immobilized probes. Hybridization conditions useful for discriminating between perfect complements and mismatches in a plethora of contexts have been described in the art. For example, hybridization conditions useful for discriminating complimentary and mismatched hybrids in a variety of SBH and other applications are described in U.S. Patent No. 5,525,464 to Drmanac *et al.*, WO 95/09248 and WO 98/31836. A particularly detailed discussion of the theoretical and practical considerations involved in determining hybridization conditions useful for SBH applications, including a discussion of the advantages of using low-temperature washing steps, may be found in WO 98/31836, particularly at pages 50-62. Additional guidance may be found in Hames and Higgins, 1985, NUCLEIC ACID HYBRIDIZATION: A PRACTICAL APPROACH, IRL Press, Oxford, England. The solution can be mixed during the hybridization, as previously described.

Following contact under conditions appropriate to the particular assay, the hybridzation solution is removed, typically by blotting with a suitable absorbent material or with the aid of a syringe or other pump. The beads of the array are then optionally washed, typically under moderate to high stringency conditions, to remove unhybridized target nucleic acid. Washing is preferably accomplished by flowing a wash solution or series of wash solutions through the linear array. The array is then scanned for signal, as previously described. Only those beads where hybridization has taken place will produce a detectable signal. Those beads containing perfectly complementary hybrids are expected to produce more intense signals than beads containing mismatched hybrids.

Depending upon the particular application, the scanned image can be used, *e.g.*, to fingerprint the target nucleic acid, to detect polymorphisms or to sequence the target nucleic acid. In this latter embodiment, the sequences of the hybridized probes are determined from their spatial addresses and their common sequences overlapped to obtain the sequence of the target nucleic acid. Computer algorithms suitable for overlapping the common sequences and a detailed theoretical discussion of SBH can be found, *e.g.*, in U.S. Patent Nos. 5,525,464 and 5,202,231.

The invention is further illustrated by the following representative examples.

7. EXAMPLE: PREPARATION OF ISOTHIOCYANATE ACTIVATED OF GLASS BEADS

In order to activate glass beads with isothiocyanate groups (-NCS) suitable for covalently attaching amino-modified polynucleotides, 4 μl of 100 μm o.d. glass beads (Duke Scientific, Inc.) were soaked in an excess volume of 1 M HCl for 16 hr. Alternatively, the beads can be soaked in 1 M nitric acid for 3 hr. The beads were then rinsed thoroughly with deionized water, followed by acetone, and allowed to air dry. The dry beads were then soaked in excess volumes of hexane, acetone and methanol, respectively, for 10 min each, and air dried. The beads must be completely dry prior to continuing with the activation procedure.

A solution containing 2% aminopropyltriethoxy silane (in 95% acetone:water) was prepared in a plastic container and allowed to stand for 10 min to activate. The dry beads were then submerged in this silane solution for about 2 min., removed, and immediately rinsed with an excess volume of acetone (3 consecutive acetone washes). The rinsed beads were allowed to air dry. The beads were cured in a dry incubator at 98° C for 45 min. and cooled for at least 10 min.

The activation solution was preared by dissolving 1,4-phenylene diisothiocyanate (PDC) in a 10% solution of pyridine:dimethyl formamide to yield a final concentration of 0.2% PDC. The cooled beads were submerge in this PDC solution and incubated for 2 hr at room temperature. The beads were then removed and washed by submersion in methanol for 5 min. followed by two successive baths of acetone for 5 min each. The isothiocyanate-activated beads were then allowed to air-dry.

8. EXAMPLE: GENE EXPRESSION

The ability to monitor gene expression with a linear array according to the invention was demonstrated using immobilized cDNAs and fluorescently-labeled oligonucleotide probes.

8.1 Construction of the Array

cDNA was covalently attached to isothiocyanate activated beads by immersing the beads in buffer (0.1 M $NaHCO_3$, pH 9) containing 5'-amino modified cDNA (0.2 $\mu g/\mu l$). Three types of beads were prepared: control beads containing no cDNA, beads containing high-expression cDNA (human serum albumin gene) and beads containing low-expression cDNA (human nucleic acid binding protein sub2.3 gene). The beads were then rinsed with water. Control beads were treated the same as beads containing cDNA, but have no DNA attached.

8.2 Preparation of Labeled Probes

Labeled probes complementary to the cDNAs were prepared by reverse transcription of a Fetal Liver and Spleen (FLS) mRNA library incorporating CY3 dye-labeled dCTP (Amersham).

8.3 Preparation and Use of Linear Array

To construct the linear array, the cDNA-coupled beads were dispensed into a 150 μm i.d. glass capillary (3.2 cm length; 550 μm o.d.; Drummond Scientific Co.) using a microfunnel similar to that illustrated in FIG. 8 with the aid of tweezers and a microscope. The ordering of the beads was as follows: 4 blank beads, 9 high-expression cDNA beads and 10 low-expression cDNA beads.

The capillary was filled with probe solution (40 μg/ml probes in 5X SSC containing 0.1% SDS) using capillary action. Hybridization was carried out overnight at 65°C in a sealed chamber. The probe solution was then removed and the array washed with 5X SSC buffer containing 0.1% SDS at room temperature with the aid of a syringe.

The array was then placed upon a glass slide and scanned for signal using a Scanarray 3000 (General Scanning) scanner

8.4 Results

The results of the assay are provided in FIG. 11. In FIG. 11, both the image of the linear array and a trace showing the signal intensities of the beads composing the array are provided. The beads containing high expression cDNA are not closely packed. Very strong signal was obtained with beads containing high-expression cDNA, whereas weak signal was obtained with the control beads and beads containing low-expression cDNA. Thus, this example demonstrates that the arrays of the invention can be used to monitor DNA expression.

9. EXAMPLE: FORMAT III SBH

This example demonstrates the ability of the linear arrays of the invention to be used in format III SBH applications which require probe hybridization and ligation.

9.1 Preparation of Array Beads

Isothiocyanate-activated beads (100 μm) were prepared as previously described. The following 5'-amino modified oligonucleotide probes were synthesized using standard techniques and covalently attached to the isothiocyanate beads, a single probe sequence per bead (5 beads each sequence), as previously described:

P1:	5' H_2N-C12-C18-GGCAT
P2:	5' H_2N-C12-C18-GCATG
P5:	5' H_2N-C12-C18-TGAAC
P6:	5' H_2N-C12-C18-GAACC
P31	5' H_2N-C12-C18-GGAGG

Following probe attachment, the beads were blocked with buffer (Liquid block 1059304, Amersham) to prevent non-specific binding of solution-phase labeled probes and template DNA.

9.2 Preparation and Use of the Linear Array

The beads were dispensed into a 150 μm i.d. capillary (3.2 cm length; 550 μm o.d.;Drummond Scientific Co.) using a microfunnel similar to that illustrated in FIG. 8 with the aid of tweezers and a microscope. Silver-coated marker beads (100 μm; Potters Industries, Inc.) were used to mark the polarity of the capillary, as well as to separate the array beads. The ordering of the beads was as follows: P31, P6, P5, P2. P1.

The linear array was filled with ligation solution by capillary action and incubated for 0.5 hr at 25 °C. The ligation solution contained 1 μg of 1μm template DNA (5' TGA GGA TGG GCC TCC AGT TCA TGC CGC CCA TGC), 5 μl of 1 μM labeled probe mixture and 400 units T4 DNA ligase dissolved in 100 μl ligation buffer (New England Biolabs). The labeled probe mixture contained the following 3'-TAMRA labeled probes:

L1:	5' GAACC
L2:	5' AACCG
L5:	5' CGGAG
L6:	5' GGAGG
L31:	5' CCCAT

After the ligation reaction, the array was washed with 0.1% SDS followed by ethanol (95 %) with the aid of a syringe. The array was then placed upon a glass slide a scanned for signal using a Scannarray 3000 scanner (General Scanning) scanner.

9.3 Results

The results of the ligation hybridization are shown in FIG. 12. A strong signal was obtained from a full ligation reaction, whereas weak signal was obtained at positions where either the immobilized oligo probe or the solution-phase labeled oligo probe contained mismatches with the template DNA.

Various embodiments of the invention have been described. The descriptions are intended to be illustrative of the present invention. It will be apparent to one of skill in the art that modifications may be made to the invention as described without departing from the scope of the claims set out below. For example, while the invention has been illustrated with particular reference to polynucleotides and nucleic acid sequencing applications, any applications utilizing spatially-addressable arrays of immobilized compounds and the particular linear arrays useful therein are contemplated to fall within the scope of the appended claims.

All references and patent sited throughout the specification herein by reference in their entireties.

What Is Claimed Is:

1. A linear array apparatus comprising a tube or channel having disposed therein a plurality individual non-porous beads aligned in a linear array, each bead having immobilized thereon a compound which is identifiable by its spatial address within the linear array.

2. The linear array apparatus of Claim 1 in which the tube is a capillary having an inner wall defining an inner lumen of diameter x and the beads are spherical and have an outer diameter smaller than x and larger than $\frac{1}{2}x$.

3. The linear array apparatus of Claim 1 in which the tube is a capillary tube having an inner diameter in the range of about 1 μm to 150 μm.

4. The linear array apparatus of Claim 1 in which and the beads are spherical and have outer diameters in the range of about 1 μm to 100 μm.

5. The linear array apparatus of Claim 1 in which at least one bead has immobilized thereon a plurality of compounds.

6. The linear array apparatus of Claim 1 in which each bead has immobilized thereon a single compound.

7. The linear array apparatus of Claim 6 in which each immobilized compound is unique.

8. The linear array apparatus of Claim 1 in which the compounds are immobilized via covalent attachment, optionally by way of a linker.

9. The linear array apparatus of Claim 8 in which the compounds are peptides.

10. The linear array apparatus of Claim 8 in which the compounds are polynucleotides.

11. The linear array apparatus of Claim 10 in which the polynucleotides are attached via a terminal nucleotide.

12. The linear array apparatus of Claim 10 in which each immobilized polynucleotide is independently 6 to 20 nucleotides in length.

13. The linear array apparatus of Claim 10 in which each immobilized polynucleotide is 6 to 20 nucleotides in length.

14. The linear array apparatus of Claim 10 in which at least one bead has a mixture of polynucleotides attached thereto.

15. The linear array apparatus of Claim 10 in which each bead has a mixture of polynucleotides attached thereto.

16. The linear array apparatus of Claim 15 in which the mixture is of the formula: $N_xB_yN_z$, where each B independently represents A, C, T, G or U and is the same for the polynucleotides in a given mixture, each N independently represents A, C, T, G or U and varies for each of the polynucleotides in a given mixture, x is an integer from 0 to 5, y is an integer from 4 to 20 and z is an integer from 0 to 5.

17. A method of sequencing a target nucleic acid, comprising the steps of:

i. contacting a linear array comprising a capillary having disposed therein a plurality of individual beads aligned in a linear array, each bead having a unique

oligonucleotide probe immobilized thereon which is identifiable by its spatial address within the linear array, with a labeled target nucleic acid under conditions which discriminate between perfect complements and mismatches between the target nucleic acid and the information content of the oligonucleotide probes;

ii. washing the linear array to remove unhybridized labeled target nucleic acid;

iii. determining which beads in the linear array hybridized with the target nucleic acid; and

iv. determining the sequence of the target nucleic acid by overlapping the common sequences of the hybridized probes.

18. A method of sequencing a target nucleic acid, comprising the steps of:

i. contacting a linear array comprising a capillary having disposed therein a plurality of individual beads aligned in a linear array, each bead having a unique oligonucleotide probe immobilized thereon which is identifiable by its spatial address within the linear array, with a target nucleic acid, a labeled oligonucleotide probe of known sequence and a DNA ligase under conditions which discriminate between perfect complements and mismatches between the target nucleic acid and the information content of the oligonucleotide probes;

ii. washing the linear array to remove unhybridized target nucleic acid and unligated labeled probe;

iii. determining which beads in the linear array ligated with the labeled probe; and

iv. determining the sequence of the target nucleic acid by overlapping the common sequences of the ligated probes.

19. The method of Claim 18 in which the labeled oligonucleotide probe comprises a plurality of unique probes bearing different distinguishable labels.

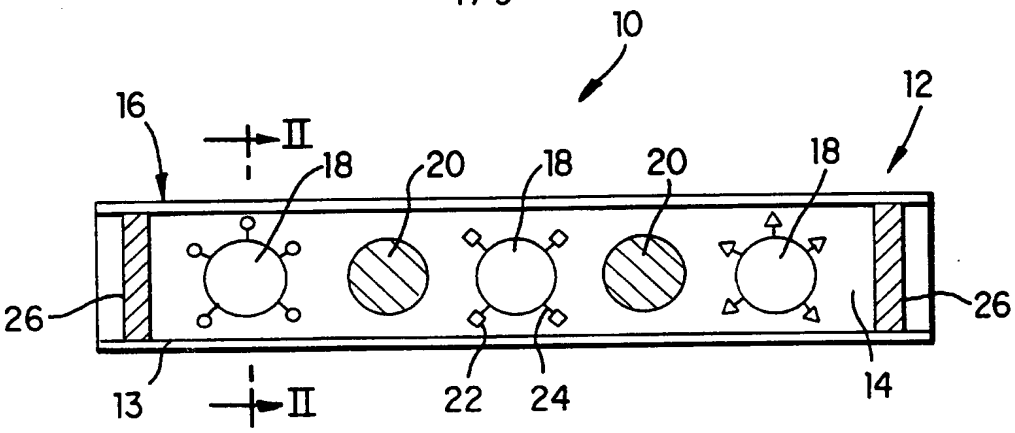

FIG. 1

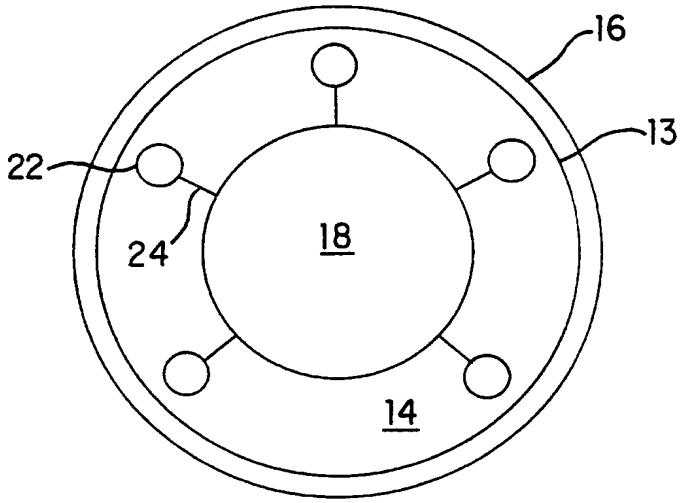

FIG. 2

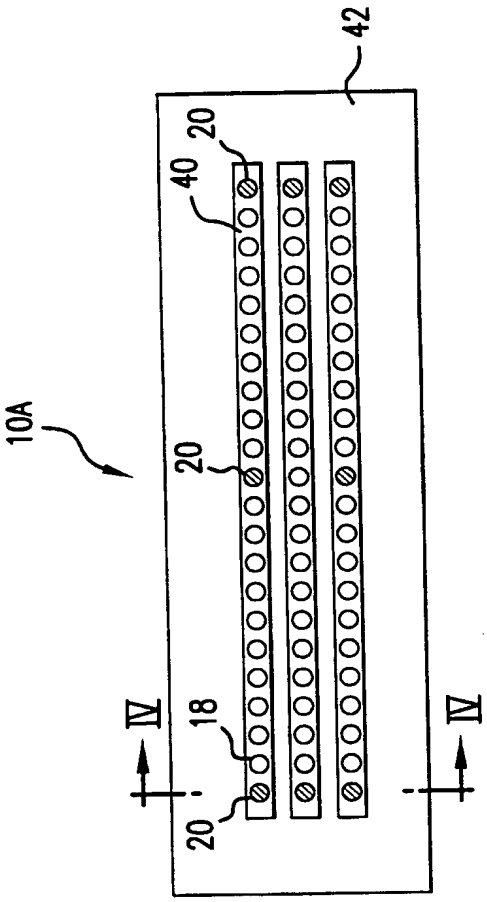

FIG.3

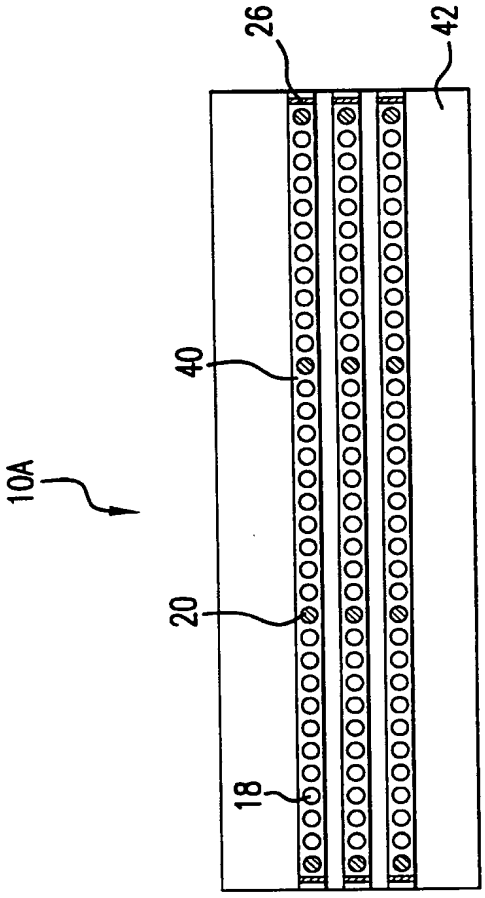

FIG.3A

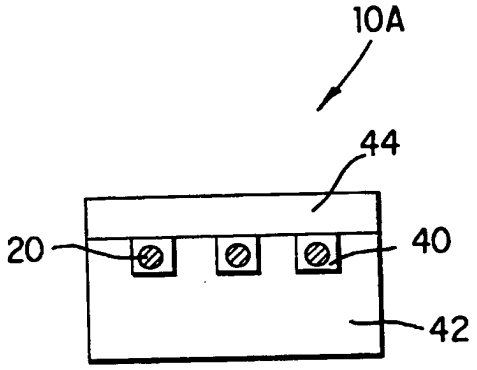

FIG. 4

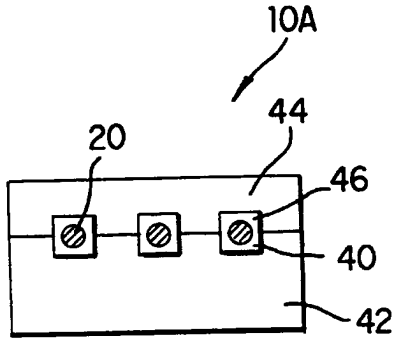

FIG. 5

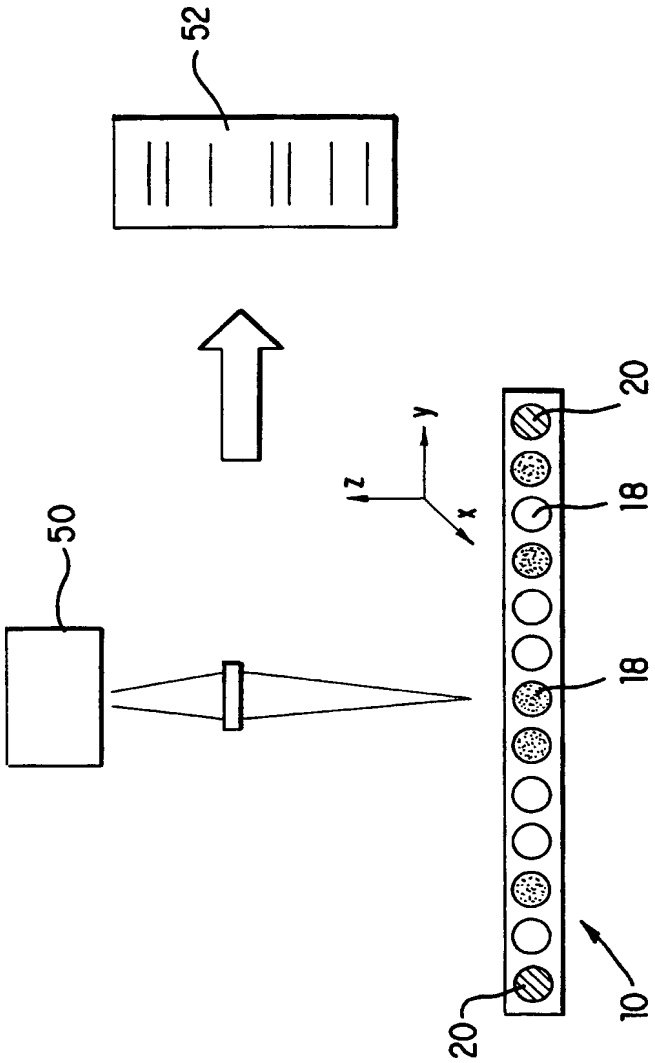

FIG. 6

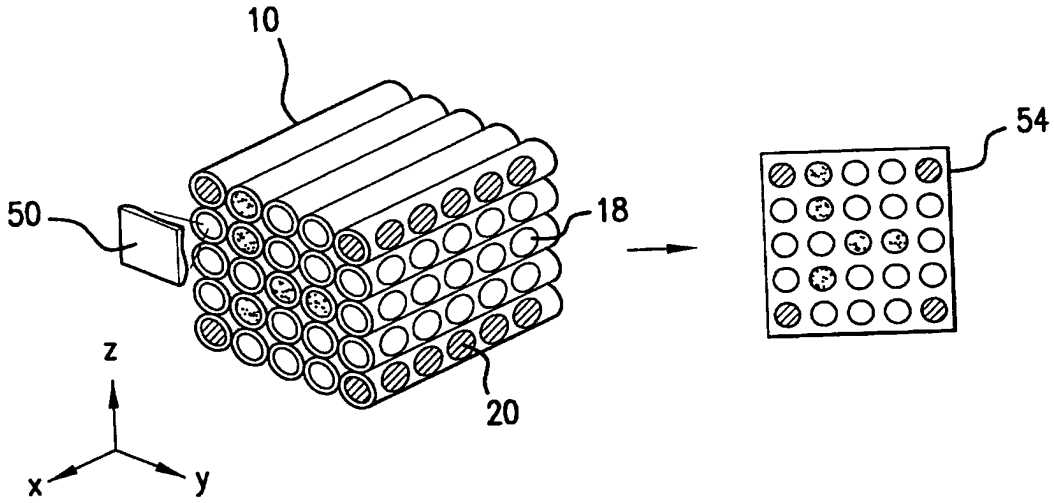

FIG.7

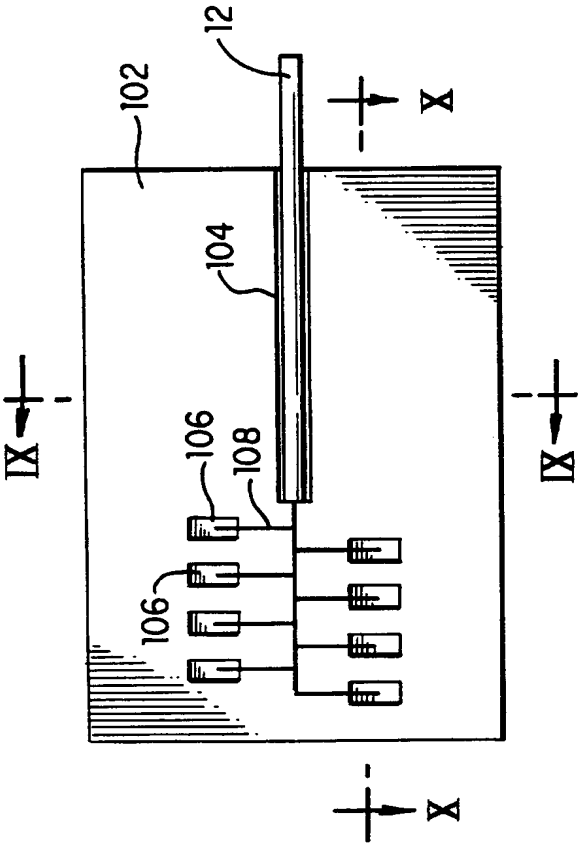

FIG. 8

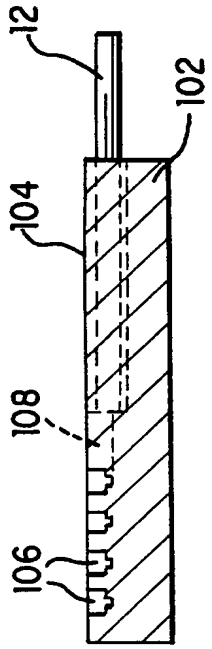

FIG. 10

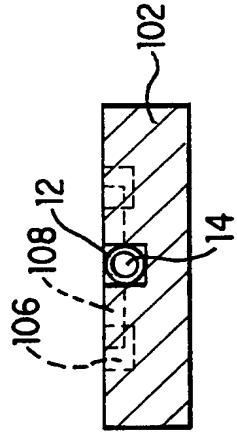

FIG. 9

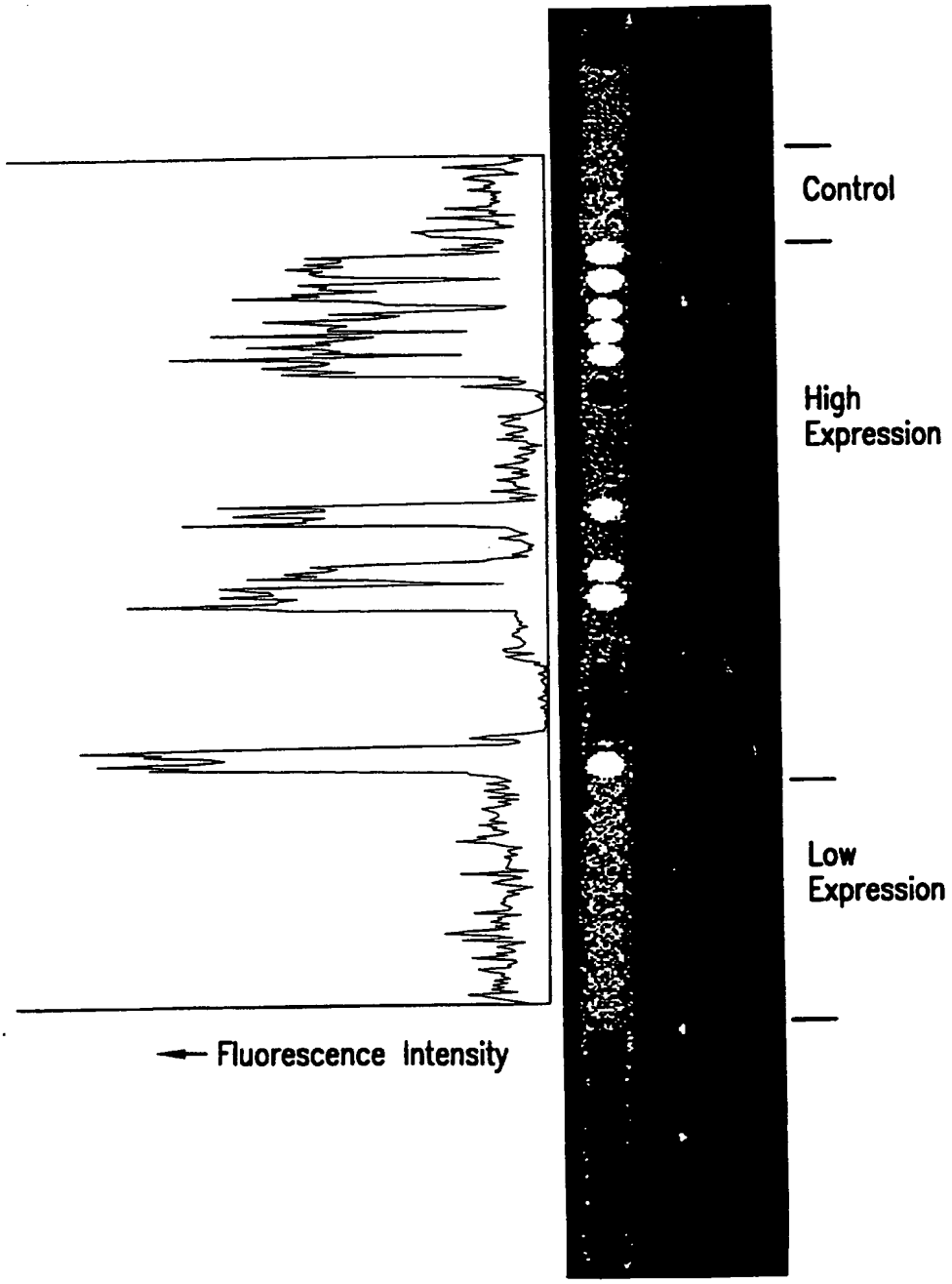
Control
High Expression
Low Expression
Fluorescence Intensity

FIG. 11

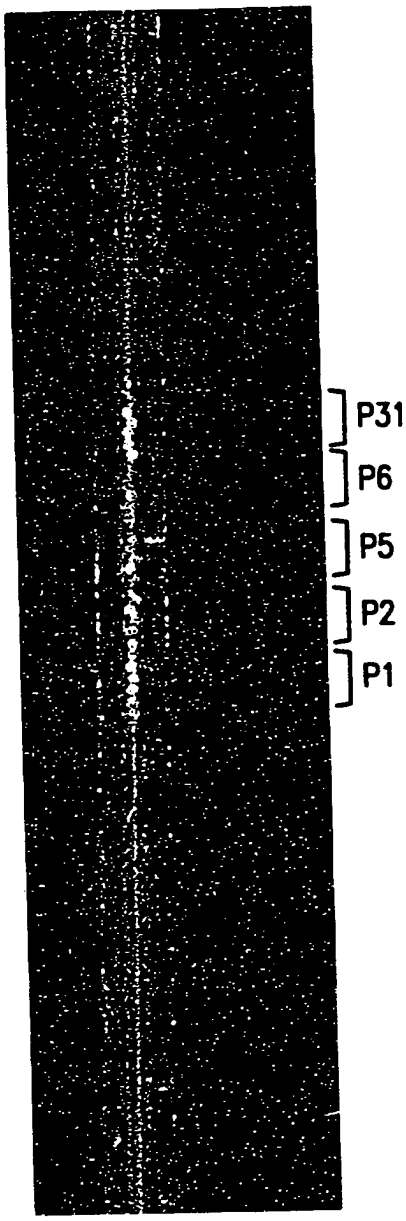

FIG.12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11216

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; C12P 19/34; C07H 21/02, 21/04, 5/04, 1/00; C08B 37/00
US CL : 435/6, 91.1, 91.52; 536/23.1, 24.32, 55.3, 56, 123.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.1, 91.52; 536/23.1, 24.32, 55.3, 56, 123.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN, caplus, medline, biosis, embase, japio, europatful
linear array, probe, hybridization, solid support, sequencing, glass beads

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,503,980 A (CANTOR) 02 April 1996, see abstract.	1-19
Y	US 5,545,531 A (RAVA et al) 13 August 1996, see abstract, especially col.1 and 2.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
15 AUGUST 1999

Date of mailing of the international search report
10 SEP 1999

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